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PART II

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Department of Health, Education, and Welfare

Food and Drug Administration

# Drugs; Tests and Methods of Assay and Certification

Notice of Proposed Rule Making

# Proposed Rule Making

## DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

### Food and Drug Administration

**[21 CFR Parts 147, 148e, 148k, 148p, 148r, 148s, 148t ]**

### ANTIBIOTICS DRUGS

### Proposed Regulations for Tests and Methods of Assay and Certification of Certain Antibiotic Drugs

The Kefauver-Harris Drug Amendments of 1962 provide that all antibiotic drugs intended for human use shall be subject to the certification provisions of sections 502(1) and 507 of the Federal Food, Drug, and Cosmetic Act. These amendments become effective May 1, 1963. Therefore, the Commissioner of Food and Drugs, pursuant to the provisions of the act (sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786; 21 U.S.C.A. 357), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (25 F.R. 8625), proposes to issue the following amendments and new regulations to provide for tests and methods of assay and certification of certain antibiotic drugs:

§ 147.1 [Amendment]

1. It is proposed to amend paragraph (e)(1) of § 147.1 *Antibiotic sensitivity discs; tests and methods of assay; potency* by changing the last sentence to read as follows: "This is the potency obtained for a single assay. Perform two or more replicate assays on each of 2 days. The average of all assays is the potency of the sample disc."

2. It is proposed to amend § 147.2 as follows:

a. By changing the introduction to paragraph (a) to read as set forth below, and by adding thereto the following new subparagraphs:

§ 147.2 Antibiotic sensitivity discs; certification procedure.

(a) *Standards of identity, strength, quality, and purity.* Antibiotic sensitivity discs are paper or plastic discs or compressed tablets containing antibiotic compounds. If they are tablets they may contain suitable lubricants, binders, and diluents, none of which shall affect the antibacterial spectrums of the antibiotics. Each disc shall have a uniform potency that is equivalent to that contained in a standard disc prepared with one of the following quantities of antibiotic compounds:

* * * * *

(12) Colistin: Not less than 2 μg or not more than 10 μg.

(13) Erythromycin: Not less than 2 μg or not more than 15 μg.

(14) Kanamycin: Not less than 5 μg or not more than 30 μg.

(15) Neomycin: Not less than 5 μg or not more than 30 μg.

(16) Novobiocin: Not less than 5 μg or not more than 30 μg.

(17) Nystatin: 100 units.

(18) Oleandomycin: Not less than 2 μg or not more than 15 μg.

(19) Oxytetracycline: Not less than 5 μg or not more than 30 μg.

(20) Polymyxin: Not less than 50 units or not more than 300 units.

(21) Ristocetin: Not less than 5 μg or not more than 30 μg.

(22) Vancomycin: Not less than 5 μg or not more than 30 μg.

(23) Viomycin: Not less than 2 μg or not more than 10 μg.

b. By changing paragraph (c)(1)(iii) (*b*) and (*c*) to read as set forth below, and by adding to subdivision (iii) a new subsection (*d*), as follows:

(c) *Labeling.* * * *

(1) * * *

(iii) * * *

(*b*) For bacitracin, nystatin, ristocetin, vancomycin, and kanamycin: 12 months.

(*c*) For streptomycin, dihydrostreptomycin, chlortetracycline, demethylchlortetracycline, tetracycline, chloramphenicol, colistin, neomycin, novobiocin, oleandomycin, oxytetracycline, polymyxin, and viomycin: 24 months.

(*d*) For erythromycine: 18 months.

§ 147.4 [Amendment]

3. It is proposed to amend § 147.4 *Antibiotic-dehydrated media-triphenyltetrazolium chloride sensitivity discs* * * * by changing paragraphs (a)(1) and (b) to read:

(a)(1) Each disc shall contain penicillin G, streptomycin, tetracycline, chloramphenicol, bacitracin, erythromycin, kanamycin, neomycin, novobiocin, oleandomycin, polymyxin B, or ristocetin. If it is tetracycline, it shall contain 5 micrograms.

* * * * *

(b) In all cases, the immediate container shall be sterile at the time of filling and closing. If it is a packaged combination, such package shall contain only those discs described in paragraph (a) of this section.

4. It is proposed to add to Title 21, Chapter I, the following new sections and parts.

§ 148e.18 Erythromycin ethylsuccinate intramuscular solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate intramuscular solution is erythromycin ethylsuccinate in a suitable and harmless solvent, with or without one or more suitable local anesthetics or preservatives. The erythromycin ethylsuccinate content is not less than 5.0 milligrams of erythromycin base per milliliter. It is sterile. It is nontoxic. Its moisture content is not more than 1.0 percent. Its pH is not less than 8.5 nor more than 9.5. The erythromycin ethylsuccinate used conforms to the requirements prescribed therefor by § 148e.7(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* (i) In addition to the labeling requirements prescribed by § 148.3 of this chapter, each immediate container shall bear on its label or labeling the following statements:

(*a*) "Warning—For intramuscular use only."

(*b*) "Do not refrigerate."

(ii) Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, moisture, identity, and crystallinity.

(*b*) The batch for potency, sterility, toxicity, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 packages, each containing 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package or immediate container submitted in accordance with subparagraph (3)(ii)(*a*), (*b*)(*1*), and (*c*) of this paragraph; $10.00 for all immediate containers submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1(b)(1)(i), except prepare the solution of the sample in the following manner: By means of a syringe, transfer 1 milliliter of the sample to a 100-milliliter volumetric flask, add 40 milliliters of absolute methyl alcohol, fill to volume and further dilute to the reference point with 0.1*M* potassium phosphate buffer, pH 8. The erythromycin ethylsuccinate content is satisfactory if it is not less than 90 percent and not more than 115 percent of the amount that it is represented to contain.

(2) *Sterility.* Use the entire contents of a single-dose ampul, or approximately 1 milliliter from each multiple-dose container and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except administer intraperitoneally a test dose of 0.08 milliliter of the undiluted solution.

(4) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter, except dissolve the sample with absolute methyl alcohol.

(5) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution.

* * * * *

**§ 148e.23 Erythromycin ethyl carbonate for pediatric drops.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethyl carbonate for pediatric drops is a dry mixture of erythromycin ethyl carbonate, with or without suspending and dispersing agents, buffer substances, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains not less than the equivalent of 100 milligrams of erythromycin base. Its moisture content is not more than 2.0 percent. Its pH is not less than 6.5 nor more than 7.0. The erythromycin ethyl carbonate used conforms to the requirements prescribed by § 148e.2(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the requirements prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethyl carbonate used in making the batch for potency, toxicity, pH, moisture, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin ethyl carbonate used in making the batch: 10 packages, each consisting of not less than 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each immediate container or package submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following procedures:

(i) *Microbiological assay.* Proceed as directed in § 148e.11(b)(1)(i).

(ii) *Spectrophotometric assay.* Reconstitute the suspension as directed in the labeling and transfer 1 milliliter of the suspension to a 1-liter volumetric flask. Dissolve the suspension with 400 milliliters of an equal-parts mixture of absolute methyl alcohol and distilled water. Proceed as directed in § 148e.1-(b)(1)(ii)(c), using the following calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{0.5 \times \text{Standard Factor K}} = \text{Milligrams of erythromycin base per milliliter.}$$

The erythromycin ethyl carbonate potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the amount it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, using 500 milligrams.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the suspension reconstituted as directed in the labeling.

## PART 148k—NYSTATIN

Sec.


148k.1 Nystatin.
148k.2 Nystatin ointment; nystatin cream.
148k.3 Nystatin-neomycin sulfate-gramicidin ointment; nystatin-neomycin sulfate-gramicidin cream.
148k.4 Nystatin topical powder.
148k.5 Nystatin-neomycin sulfate-gramicidin topical powder.
148k.6 Nystatin for oral suspension.
148k.7 Nystatin tablets.
148k.8 [Reserved]
148k.9 [Reserved]
148k.10 Nystatin-neomycin sulfate-polymyxin B sulfate for otic solution.


AUTHORITY: §§ 148k.1 to 148k.10 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

**§ 148k.1 Nystatin.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin is the yellow to light-tan compound of a kind of nystatin or a mixture of two or more such compounds. It is very slightly soluble in water, moderately soluble in methyl alcohol, butyl alcohol, or propyl alcohol. It is so purified and dried that:

(i) Its potency is not less than 2,000 units per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5.0 percent.

(iv) Its pH in a 3 percent aqueous suspension is not less than 6.5 and not more than 8.0.

(v) It exhibits absorption maxima within ±2 millimicrons at 230 millimicrons, 291 millimicrons, 305 millimicrons, and 319 millimicrons when dissolved in methyl alcohol, and the ratio of the absorption peak at 230 millimicrons ±2 millimicrons and the middle of the shoulder at 279 millimicrons ±2 millimicrons, $\frac{A_{230}}{A_{279}}$, is not less than 0.90 and not more than 1.25.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 300 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay*—(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F.

(*1*) Make nutrient agar for carrying the organism as follows:

| | |
|---|---|
| Peptone | 6.0 gm. |
| Pancreatic digest of casein | 4.0 gm. |
| Yeast extract | 3.0 gm. |
| Beef extract | 1.5 gm. |
| Glucose | 1.0 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 6.5 to 6.6 after sterilization.

(*2*) Make nutrient agar for preparing the inoculated agar plates, as follows:

| | |
|---|---|
| Peptone | 9.4 gm. |
| Yeast extract | 4.7 gm. |
| Beef extract | 2.4 gm. |
| Sodium chloride | 10.0 gm. |
| Dextrose | 10.0 gm. |
| Agar | 23.5 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 6.0 to 6.2 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*c*) *Working standard.* Dry an appropriate amount of the working standard for 2 hours at 40° C. at a pressure of 5 millimeters or less. Determine the dry weight and dissolve in sufficient dimethyl formamide to give a stock solution of convenient concentration (usually 1,000 units to 5,000 units per milliliter). This stock solution should be prepared simultaneously with the samples to be tested and should be used for 1 day only.

(*d*) *Preparation of sample.* Dissolve the sample to be tested in sufficient dimethyl formamide to give a nystatin concentration of 400 units per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter (estimated).

(*e*) *Preparation of test organism.* The test organism is *Saccharomyces cerevisiae* (ATCC 9763 or ATCC 2601, no vitamin requirement), which is maintained on slants of agar described in (b)(*1*) of this subdivision. Wash the organism from the agar slant with 3 milliliters of sterile distilled water onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in (b)(*1*) of this subdivision. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C. and then wash the resulting growth from the agar surface with about 30 milliliters of sterile distilled water. Standardize the suspension so that a 1:45 dilution of it will give 25

percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of a 1:12 dilution in sterile distilled water that should be added (usually 0.7 milliliter) to each 100 milliliters of agar to give clear, sharp inhibition zones of appropriate size.

(*f*) *Preparation of plates.* Melt a sufficient amount of the agar described in (*b*) (*2*) of this subdivision, cool to 48° C., add the proper amount of the test organism as described in (*e*) of this subdivision, and mix thoroughly. Add 8 milliliters of this inoculated agar to each Petri dish (100 millimeters x 22 millimeters, with flat bottom). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Dilute aliquots of the standard stock solution with dimethyl formamide to give concentrations of 256, 320, 400, 500, and 624 units per milliliter. Dilute these solutions with 10 percent potassium phosphate buffer, pH 6.0, to make concentrations of 12.8, 16.0, 20.0, 25.0, and 31.2 units per milliliter, respectively. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 20 units per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with the 20 units per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be 36 twenty-unit determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 37° C. and measure the diameter of each zone of inhibition. Average the readings of the 20 units per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 20 units per milliliter concentration. The average of the 36 readings of the 20 units per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 20 units per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 16 units per milliliter concentration, the average of the 36 readings of the 20 units per milliliter concentration is 16.0 millimeters and the average of the 20 units per milliliter concentration of this set of three plates is 15.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 16 units per milliliter concentration of those same three plates is 15.0 millimeters, the corrected value is then 15.2 millimeters. Plot these corrected values, including the average of the 20 units per milliliter concentration, on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

$L$ = Corrected zone diameter for the lowest concentration of the standard curve;

$H$ = Corrected zone diameter for the highest concentration of the standard curve;

$c$ = Average zone diameter of 36 readings of the 20 units per milliliter standard;

$a, b, d, e$ = Corrected average values for the 12.8, 16.0, 25.0, and 31.2 units per milliliter standard solutions, respectively.

Plot the values obtained for $L$ and $H$ and connect with a straight line.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 20 units per milliliter standard and three cylinders with the 20 units per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 37° C. and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 20 units per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 20 units per milliliter value on the curve. From the curves, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay—(a) Culture medium.* Make nutrient broth for preparing an inoculum of the test organism as follows:

| | |
|---|---|
| Peptone | 10 gm. |
| Dextrose | 20 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 5.6 to 5.7 after sterilization.

(*b*) *Working standard and standard solutions.* Prepare the standard stock solution as directed in subdivision (i) (*c*) of this subparagraph. Make dilutions of the stock solution with dimethyl formamide to obtain concentrations of 121, 139, 184, and 212 units per milliliter. These solutions are then diluted 1:10 with sterile distilled water to obtain concentrations of 12.1, 13.9, 16.0, 18.4, and 21.2 units per milliliter. Add 1.0 milliliter of each such concentration to each of six sterile 20 millimeters x 150 millimeters test tubes. Maintain the sterility of the tubes.

(*c*) *Preparation of sample.* Dissolve the sample to be tested in sufficient dimethyl formamide to give a stock solution of convenient concentration. Dilute the stock solution with dimethyl formamide to obtain a concentration of 160 units per milliliter (estimated). Further dilute this solution 1:10 with sterile distilled water to obtain a concentration of 16 units per milliliter (estimated). Add 1.0 milliliter of this dilution to each of six sterile 20 millimeters x 150 millimeters test tubes.

(*d*) *Preparation of test organism.* Maintain the test organism *Saccharomyces cerevisiae* (ATCC 9763) on agar slants as described in subdivision (i) (*e*) of this subparagraph. For use in the assay, prepare an organism suspension by inoculating 100 milliliters of the medium described in (*a*) of this subdivision with a loopful of growth from an agar slant, and incubate the broth culture overnight at 37° C. Adjust this stock suspension, so that a 1:10 dilution in the nutrient broth will have an absorption of 0.350 at a wavelength of 650 millimicrons, using a suitable photoelectric colorimeter and an 18-millimeter diameter test tube as an absorption cell. Prepare the inoculum broth by adding from 3 milliliters to 5 milliliters of that adjusted suspension (not the 1:10 dilution) to each liter of nutrient broth needed for the test.

(*e*) *Procedure.* To each of the 20 millimeters x 150 millimeters test tubes prepared in (*b*) and (*c*) of this subdivision, add 9.0 milliliters of the inoculated nutrient broth described in (*d*) of this subdivision, and incubate at 26° C. for 16 hours to 18 hours. After incubation, add 0.5 milliliter of a 12 percent formaldehyde solution to each tube and read the absorbance values in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear, uninoculated broth prepared as described in (*a*) of this subdivision.

(*f*) *Estimation of potency.* Plot the average values for each concentration of the standard on semilog graph paper with absorbance values on the arithmetic scale and nystatin concentrations on the logarithmic scale. Construct the best straight line through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

$L$ = Absorbance value for the lowest concentration of the standard curve;

$H$ = Absorbance value for the highest concentration of the standard curve;

$a, b, c, d, e$ = Average absorbance values for each concentration of the standard curve.

Plot the values obtained for $L$ and $H$ and connect the points with a straight line. Average the absorbance values for the sample and read the nystatin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the nystatin content of the sample.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except inject intraperitoneally each of 5 mice with 0.5 milliliter of a suspension of the drug containing 1,200 units per milliliter in a 0.5 percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 3 percent aqueous suspension of the drug.

(5) *Identity.* Weigh approximately 20 milligrams of the sample in a 100-milliliter glass-stoppered volumetric flask, add about 75 milliliters absolute methyl alcohol and shake mechanically for 30 minutes. Dilute to volume with methyl alcohol. Transfer 10.0 milliliters of this solution to a 100-milliliter volumetric flask and dilute to volume with methyl alcohol. Within 2 hours, determine the absorption peak at 230 millimicrons, 291 millimicrons, 305 millimicrons, and 319 millimicrons, and the shoulder at 279±2 millimicrons, using a suitable ultraviolet spectrophotometer and quartz cells. Set the instrument to 100 percent transmission with absolute methyl alcohol. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 220 millimicrons to 320 millimicrons. If a nonrecording spectrophotometer is used, the exact positions of the peaks and shoulder should be determined for the particular instrument used. The ratio of the two absorbances $\left(\frac{A_{230}}{A_{279}}\right)$ should be not less than 0.90 and not more than 1.25.

**§ 148k.2 Nystatin ointment; nystatin cream.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin ointment or nystatin cream is nystatin, with or without suitable and harmless preservatives, diluents, and emulsifers, suspended or dissolved in a suitable ointment or cream base. Its potency is not less than 100,000 units per gram. The moisture content of the ointment is not more than 1.0 percent. The nystatin used conforms to the requirements of § 148k.1(a)(1)(i), (iii), (iv), and (v). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency (and moisture, if it is the ointment).

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 containers, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used: One package each consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Blend for 3 minutes an accurately weighed representative portion (usually 1.0 gram) in a high-speed glass blender with sufficient dimethyl formamide to give a concentration of 400 units per milliliter (estimated). Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter, and proceed as directed in § 148k.1(b)(1). Its nystatin content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

**§ 148k.3 Nystatin-neomycin sulfate-gramicidin ointment; nystatin-neomycin sulfate-gramicidin cream.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin ointment or nystatin-neomycin sulfate-gramicidin cream is nystatin, neomycin sulfate, and gramicidin, in a suitable and harmless ointment or cream base. The drug may contain one or more suitable preservatives. Each gram contains not less than 100,000 units of nystatin, not less than 2.5 milligrams of neomycin, and not less than 0.25 milligram of gramicidin. Its moisture content is not more than 1.0 percent, if it is the ointment. The nystatin used conforms to the requirements of § 148k.1(a)(1)(i), (iii), (iv), and (v). The neomycin sulfate used conforms to the requirements of § 148i.1(a)(1)(i), (vi), (vii), and (viii) of this chapter. The gramicidin used conforms to the requirements of § 148f.1(a)(1)(i), (iii), and (iv) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, moisture, and identity.

(*d*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 500 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of 8 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used; one package each consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each container submitted in accordance with subparagraph (3)(ii)(*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 148k.2(b)(1). Its nystatin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units per gram that it is represented to contain.

(ii) *Neomycin content.* Proceed as directed in § 148f.3(b)(1) of this chapter. Its neomycin sulfate content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

(iii)(*a*) *Gramicidin content of the nystatin-neomycin sulfate-gramicidin ointment.* Proceed as directed in § 148f.1(b)(1) of this chapter, except prepare the sample in the following manner: Accurately weigh and dissolve approximately 2 grams of the sample in 50 milliliters of *n*-hexane in a separatory funnel. Extract with 50 milliliters of absolute ethyl alcohol. Repeat the extraction three times. Pool the extractives and bring to a volume of 500 milliliters with 95 percent ethyl alcohol.

(*b*) *Gramicidin content of the nystatin-neomycin sulfate-gramicidin cream.* Proceed as directed in § 148f.1(b)(1) of this chapter, except prepare the sample in the following manner: Blend 2 grams of the sample, accurately weighed, in a high-speed, glass blender, using 500 milliliters of 95 percent alcohol. Its gramicidin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

**§ 148k.4 Nystatin topical powder.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin topical powder is nystatin, with or without suitable and harmless preservatives, diluents, lubricants, and local anesthetics. Each gram contains not less than 100,000 units of nystatin. Its moisture content is not more than 2.0 percent. The nystatin used used conforms to the requirements of § 148k.1(a)(1)(i), (iii), (iv), and (v). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(c) In case of an initial request for certification, each other ingredient used: One container of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148k.1(b) (1), except prepare the sample in the following manner: Blend the entire contents of an accurately weighed representative sample for 2 to 3 minutes in a high-speed, glass blender with 500 milliliters of dimethyl formamide. Dilute with sufficient dimethyl formamide to yield a stock solution containing 400 units of nystatin per milliliter. The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units of nystatin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

**§ 148k.5 Nystatin-neomycin sulfate-gramicidin topical powder.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-gramicidin topical powder is a dry powder composed of nystatin, neomycin sulfate, and gramicidin, with or without one or more suitable and harmless preservatives, diluents, lubricants, and local anesthetics. Each gram contains not less than 100,000 units of nystatin, not less than 2.5 milligrams of neomycin and not less than 0.25 milligram of gramicidin. Its moisture content is not more than 2.0 percent. The nystatin used conforms to the requirements of § 148k.1(a) (1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the requirements of § 148i.1 (a) (1) (i), (vi), (vii), and (viii) of this chapter. The gramicidin used conforms to the requirements of § 148f.1(a) (1) (i), (iii), and (iv) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, moisture, and identity.

(*d*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of 500 milligrams.

(*c*) The gramicidin used in making the batch: Six packages, each consisting of 500 milligrams.

(*d*) The batch: A minimum of eight immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used: One package each consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each container submitted in accordance with subparagraph (3) (ii) (*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin content.* Proceed as directed in § 148k.4(b) (1). The nystatin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units per gram that it is represented to contain.

(ii) *Neomycin content.* Proceed as directed in § 148i.1(b) (1) of this chapter, except prepare the sample in the following manner: Blend the entire contents of an accurately weighed representative sample in 500 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0. The neomycin sulfate content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 148f.1(b) (1) of this chapter, except prepare the sample in the following manner: Dissolve the entire contents or a 1-gram sample in absolute ethyl alcohol, filter and proceed, using the filtrate. The gramicidin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams of gramicidin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

**§ 148k.6 Nystatin for oral suspension.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin for oral suspension is a dry powder consisting of nystatin, with or without suitable and harmless buffering and suspending substances, preservatives, diluents, colorings, and flavorings. When the suspension is prepared as directed in its labeling each milliliter contains not less than 25,000 units of nystatin. The pH of the reconstituted suspension is not less than 4.5 and not more than 5.5. Its moisture content is not more than 7.0 percent. The nystatin used conforms to the requirements of § 148k.1(a) (1). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used: One container each consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute the drug as directed in the labeling. Blend an appropriate aliquot (usually from 1 milliliter to 5 milliliters) in a high-speed, glass blender for 2 to 3 minutes, using 200 milliliters of dimethyl formamide. Dilute an aliquot with sufficient dimethyl formamide to give a stock solution containing 400 units of nystatin per milliliter. Proceed as directed in § 148k.1 (b) (1). Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units of nystatin that it is represented to contain.

(2) *Moisture.* Using the dry powder, proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the suspension reconstituted as directed in the labeling.

**§ 148k.7 Nystatin tablets.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin tablets are tablets composed of nystatin, with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains not less than 100,000 units of nystatin. The moisture content is not more than 5 percent. The nystatin used conforms to the requirements of § 148k.1(a) (1). Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(2) For disintegration time studies: Six tablets.

(c) In case of an initial request for certification, each other ingredient used: One container of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3) (ii) (*b*) (2) of this paragraph; $0.75 for each tablet submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148k.1 (b) (1), except prepare the sample in the following manner: Blend a representative number of tablets for 2 to 3 minutes in a high-speed, glass blender with 500 milliliters of dimethyl formamide. Dilute an aliquot with sufficient dimethyl formamide to give a stock solution containing 400 units of nystatin per milliliter. The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§§ 148k.8, 148k.9 [Reserved]

**§ 148k.10 Nystatin-neomycin sulfate-polymyxin B sulfate for otic solution.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Nystatin-neomycin sulfate-polymyxin B sulfate for otic solution is a dry mixture of nystatin, neomycin sulfate, and polymyxin B sulfate, with or without cortisone or a suitable derivative of cortisone. Its moisture content is not more than 6.5 percent. It may be packaged in combination with an immediate container of a suitable and harmless aqueous diluent, containing suitable preservatives and buffers. When reconstituted as directed in the labeling, each milliliter contains not less than 100,000 units of nystatin, 3.5 milligrams of neomycin base, and 10,000 units of polymyxin B sulfate. Its pH is not less than 5.5 and not more than 7.5. The nystatin used conforms to the requirements of § 148k.1(a) (1) (i), (iii), (iv), and (v). The neomycin sulfate used conforms to the requirements of § 148i.1(a) (1) (i), (vi), (vii), and (viii) of this chapter. The polymyxin B sulfate used conforms to the requirements of § 148p.1(a) (1) (i), (iii), (iv), and (v) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The nystatin used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*d*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The nystatin used in making the batch: 10 packages, each consisting of not less than 500 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each consisting of not less than 500 milligrams.

(*c*) The polymyxin B sulfate used in making the batch: 10 packages, each consisting of not less than 500 milligrams.

(*d*) The batch: A minimum of 8 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One container of each consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each container submitted in accordance with subparagraph (3) (ii) (*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Nystatin.* Proceed as directed in § 148k.6(b) (1). Its nystatin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(ii) *Neomycin sulfate.* Proceed as directed in § 148i.1(b) (1) of this chapter, using the suspension reconstituted as directed in the labeling. The neomycin sulfate content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(iii) *Polymyxin B sulfate.* Proceed as directed in § 148p.1(b) (1) of this chapter, using the suspension reconstituted as directed in the labeling. The polymyxin B sulfate content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter, using the dry powder.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the drug reconstituted as directed in its labeling.

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## PART 148p—POLYMYXIN

Sec.


148p.1 Polymyxin B sulfate.
148p.2 Polymyxin B sulfate tablets.
148p.3 Polymyxin B sulfate ointment.
148p.4 Polymyxin B sulfate otic solution.
148p.5 Polymyxin B sulfate urethral ointment.
148p.6 Polymyxin B sulfate for intramuscular use.


AUTHORITY: §§ 148p.1 to 148p.6 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

**§ 148p.1 Polymyxin B sulfate.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 7.0 percent.

(vii) Its pH in an aqueous solution containing 50,000 units per milliliter is not less than 5.0 and not more than 7.5.

(viii) Its ash content is not more than 5 percent.

(ix) If it is intended for systemic medication, its heavy-metals content is not more than 30 parts per million.

(x) It gives positive color identity tests for polymyxin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, histamine, moisture, pH, ash content, and heavy metals.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing not less than approximately 500 milligrams.

(4) *Fees.* $10.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) (*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3) (ii) (*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media.* Using ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F., make nutrient agar for carrying the test organism, and for the seed and base layers:

(*a*) For carrying the test organism:

| | |
|---|---|
| Peptone | 6.0 gm. |
| Pancreatic digest of casein | 4.0 gm. |
| Yeast extract | 3.0 gm. |
| Beef extract | 1.5 gm. |
| Glucose | 1.0 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.00 ml. |

pH 6.5 to 6.6 after sterilization.

(*b*) Base layer:

| | |
|---|---|
| Pancreatic digest of casein | 17.0 gm. |
| Papaic digest of soybean | 3.0 gm. |
| Sodium chloride | 5.0 gm. |
| Dipotassium phosphate | 2.5 gm. |
| Dextrose | 2.5 gm. |
| Agar | 20.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

Final pH 7.3.

(c) Seed layer: For the seed layer, make the following changes in the medium described in (b) of this subdivision.

| | |
|---|---|
| Agar | 12.0 gm. |
| Polysorbate 80 (added after boiling) | 10.0 gm. |

Final pH 7.3.

In lieu of preparing the medium from the individual ingredients as specified, is may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the specified individual ingredients are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(iii) *Working standard.* Dry approximately 30 milligrams of the standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine accurately the dry weight, dissolve in 2.0 milliliters of distilled water, and then add sufficient 10 percent potassium phosphate buffer, pH 6.0, to make a 1,000 units per milliliter stock solution. This solution may be used for 2 weeks if kept in refrigerator.

(iv) *Preparation of sample.* Dissolve the sample in sufficient 10 percent potassium phosphate buffer (pH 6.0) to give a convenient stock solution. Further dilute in 10 percent potassium phosphate buffer, pH 6.0, to give a final concentration of 10 units per milliliter (estimated) of polymyxin B base.

(v) *Preparation of test organism.* The test organism is *Bardetella bronchiseptica* (ATCC 4617), which is maintained on slants of agar described in subdivision (ii) (a) of this subparagraph. Wash the organism from the agar slant with 3 milliliters of sterile sodium chloride solution onto a large surface such as that provided by a Roux bottle containing 300 milliliters of agar described in subdivision (ii) (a) of this subparagraph. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface with 50 milliliters of sodium chloride solution. Standardize the suspension, so that a 1:20 dilution will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the 1:20 dilute suspension (usually 0.3 percent) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) (b) of this subparagraph to each Petri dish (20 millimeters by 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow it to harden. Use plates the same day they are prepared. Melt a sufficient amount of the agar described in subdivision (ii) (c) of this subparagraph, cool to 48° C.–50° C., add the proper amount of test organism as described in subdivision (v) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 units per milliliter working standard stock solution in 10 percent potassium phosphate buffer, pH 6.0, to obtain concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 units per milliliter. Use three plates for the determination of each point on the curve, except the 10.0 units per milliliter concentration, a total of 12 plates. On each of three plates, fill three cylinders with the 10.0 units per milliliter standard, and the other three cylinders with the concentration under test. Thus, there will be thirty-six 10.0-unit determinations and nine determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 10.0 units per milliliter and the readings of the points tested for each set of three plates, and average also all 36 readings of the 10.0 units per milliliter concentration. The average of the 36 readings of the 10.0 units per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 10 units per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 8.0 units per milliliter concentration, the average of the 36 readings of the 10-unit concentration is 20.0 millimeters and the average for the 10-unit concentration of this set of three plates is 19.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 8.0-unit concentration of these same three plates is 19.0 millimeters, the corrected value is 19.2 millimeters. Plot these corrected values, including the average of the 10 units per milliliter concentration, on semilog paper, placing the concentration in units per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

$L$ = Corrected zone diameter for the lowest concentration of the standard curve;
$H$ = Corrected zone diameter of the highest concentration of the standard curve;
$c$ = Average zone diameter of 36 readings of the 10 units per milliliter standard;
$a$, $b$, $d$, $e$ = Corrected values for the 6.4, 8.0, 12.5, and 15.6 units per milliliter standard solutions, respectively.

Plot the values obtained for $L$ and $H$ and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 10 units per milliliter solution and three cylinders with the 10 units per milliliter (estimated) sample, alternating sample and standard. Incubate all plates, including those containing the standard curve, for 16 hours to 18 hours at 37°C. Measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 10 units per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 10 units per milliliter value on the curve. Read the potencies corresponding to these corrected values of zone size.

(2) *Sterility.* Proceed as directed in § 141a.2 of this chapter, except use not less than 500 milligrams (activity) in each tube, and except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, except use as a test dose 1.0 milliliter per kilogram of solution containing 20,000 units per milliliter in sterile, pyrogen-free saline T.S.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution containing 1,200 units per milliliter in sterile saline T.S.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter of a solution containing 5 milligrams (activity) per milliliter in sterile saline T.S.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, except use a solution containing 50,000 units per milliliter.

(8) *Ash content.* Proceed as directed in § 141e.401(g) of this chapter.

(9) *Heavy-metals content.* Proceed as directed in § 148m.1(b)(10) of this chapter.

(10) *Identity.* (i) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine, boil for 1 minute, and cool; a blue color develops.

(ii) To a solution of 2 milligrams of polymyxin B sulfate in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10), mix well, and add, dropwise, 5 drops of a cupric sulfate solution (1:100), mixing after the addition of each drop; a reddish-violet color is produced.

**§ 148p.2 Polymyxin B sulfate tablets.**

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate tablets are tablets that contain polymyxin B sulfate, with or without one or more suitable and harmless diluents, binders, lubricants, colorings, and flavorings. Each tablet contains not less than 250,000 units of polymyxin B sulfate. The moisture content is not more than 40 percent. Tablets not exceeding 15

millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The polymyxin B sulfate used conforms to the requirements of § 148p.1(a)(1) (i), (iv), (vi), (vii) and (x). Each other substance used, if its name appears in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The polymyxin B used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum of 30 tablets.

(*2*) For disintegration time studies: Six tablets.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each tablet in the sample submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph; $3.00 for all tablets in the sample submitted in accordance with subparagraph (3) (ii) (*b*) (2) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.1 (b) (1), except prepare the sample by blending a representative number of tablets (usually 5 to 12) in a high-speed glass blender with an appropriate amount of 10 percent potassium phosphate buffer, pH 6.0, to prepare a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot in 10 percent potassium phosphate buffer, pH 6.0, to a final concentration of 10 units of polymyxin activity (estimated) per milliliter. The potency of each tablet is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units of activity that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

**§ 148p.3 Polymyxin B sulfate ointment.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate ointment is polymyxin B sulfate in a suitable and harmless ointment base. Its potency is not less than 10,000 units per gram. Its moisture content is not more than 1.0 percent. The polymyxin B sulfate used conforms to the standards prescribed therefor by § 148p.1 (a) (1) (i), (vi), (vii), and (x). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The polymyxin B used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.1 (b) (1), except prepare the sample by accurately weighing a representative portion of the ointment (usually 1.0 gram) and transferring it to a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake with four 25-milliliter potions of 10 percent potassium phosphate buffer, pH 6.0, and combine the aqueous extractives. After adjusting the volume of the combined extractives to 100 milliliters with 10 percent potassium phosphate buffer, pH 6.0, remove an aliquot and further dilute with 10 percent potassium phosphate buffer, pH 6.0, to obtain a concentration of 10 units (estimated) per milliliter. However, if the ointment has a water-soluble base, accurately weigh approximately 5 grams and place in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a final volume of 200 milliliters. Using a high-speed blender, blend the mixture for 2 minutes and then make the proper estimated dilutions, using 10 percent phosphate buffer, pH 6.0. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units per gram that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

**§ 148p.4 Polymyxin B sulfate otic solution.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfateotic solution is polymyxin B sulfate with or without cortisone or a suitable derivative of cortisone, and one or more suitable and harmless preservatives and buffer substances, suspended or dissolved in a suitable and harmless vehicle. Each milliliter contains not less than the equivalent of 5,000 units of polymyxin activity. Its pH is not less than 3.7 and not more than 5.0. The polymyxin B sulfate used conforms to the requirements of § 148p.1(a)(1) (i), (vi), (vii), and (x). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) Polymyxin B used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.1(b) (1), except prepare the sample by diluting volumetrically a representative sample (usually 1.0 milliliter) in 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Further dilute volumetrically in aliquot in 10 percent potassium phosphate buffer, pH 6.0, to give a final concentration of 10 units of polymyxin activity (estimated) per milliliter. Its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units of activity that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution.

**§ 148p.5 Polymyxin B sulfate urethral ointment.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate urethral ointment is polymyxin B sulfate, with or without one or more suitable and harmless dispersing, suspending, stabilizing, and preservative agents, in a suitable and harmless ointment base. It may contain a suitable local antiseptic. The potency is not less than 5,000 units of polymyxin activity per gram. It is sterile. Its moisture content is not more than 1.0 percent. The polymyxin B sulfate used conforms to the requirements of § 148p.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 packages.

(2) For sterility testing: 10 packages.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch; one package of each containing equal portions of approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*)(*1*), and (*c*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*)(2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148p.3(b)(1). Its content of polymyxin is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 148p.1(b)(2), using approximately ½ gram of the ointment for testing.

(3) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

### § 148p.6 Polymyxin B sulfate for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate for intramuscular use is polymyxin B sulfate with or without a suitable local anesthetic and with or without one or more suitable and harmless buffer substances and preservatives. Its potency is not less than 500,000 units of polymyxin activity. It is sterile. It is nonpyrogenic. It is nontoxic. It contains no histamine nor histamine-like substances. The moisture content is not more than 4.0 percent. Its pH is not less than 5.0 and not more than 7.0. The polymyxin B sulfate used conforms to the requirements of § 148p.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, pyrogens, toxicity, histamine, moisture, and pH.

(ii) Samples required:

(*a*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification each other ingredient used in making the batch: One package of each containing not less than approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*)(*1*), and (c) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii) (*b*)(2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute as directed on the label, remove a representative portion with a suitable syringe, place into an appropriate sized volumetric flask and proceed as directed in § 148p.1(b)(1). Its content of polymyxin B sulfate is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of units that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 148p.1(b)(2).

(3) *Pyrogens.* Proceed as directed in § 148p.1(b)(3).

(4) *Toxicity.* Proceed as directed in § 148p.1(b)(4).

(5) *Histamine.* Proceed as directed in § 148p.1(b)(5).

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the drug reconstituted as directed in the labeling.

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## PART 148r—TYROTHRICIN

Sec.

148r.1 Tyrothricin.

AUTHORITY: § 148r.1 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

### § 148r.1 Tyrothricin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tyrothricin is a white to brownish-white crystalline compound of a kind of tyrothricin or a mixture of two or more such compounds. It consists principally of gramicidin and tyrocidine. It is so purified and dried that:

(i) Its potency is not less than 180 micrograms per milligram in terms of gramicidin master standard.

(ii) Its moisture content is not more than 5 percent.

(iii) It gives a positive identity test for tyrothricin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b). Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, crystallinity, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Tyrothricin is assayed, using the gramicidin working standard. Proceed as directed in § 148g.1(b)(1) of this chapter, except dilute the tyrothricin sample to a final estimated concentration of 0.20 microgram per milliliter. Average the absorbance values for the tyrothricin sample, and read the gramicidin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the gramicidin content of the sample. Further multiply by a factor of 5 to obtain the amount of tyrothricin in the sample.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(4) *Identity.* To 5 milliliters of *p*-dimethylaminobenzaldehyde (T.S.) add about 5 milligrams of tyrothricin, shake well for 2 minutes, then add 2 drops of a 0.1*M* sodium nitrite and 5 milliliters of water. A blue color is produced.

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## PART 148s—VANCOMYCIN

Sec.

148s.1 Vancomycin hydrochloride.

148s.2 Vancomycin hydrochloride for intravenous use.

AUTHORITY: §§ 148s.1 to 148s.2 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785 786, 787; 21 U.S.C.A. 357.

### § 148s.1 Vancomycin hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride is the crystalline hydrochloride salt of a kind of vancomycin or a mixture of two or more such salts. It is soluble in water and moderately soluble in dilute methyl alcohol. It is insoluble in higher alcohols, acetone, and ether. It is so purified and dried that:

(i) It contains not less than 900 micrograms of vancomycin activity per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 5 percent.

(vii) Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5.

(viii) It contains not more than 15 percent of Factor A.

(ix) It gives a positive identity test for vancomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of

§ 148.3(b). Its expiration date is 12 months

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, crystallinity, Factor A content, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media.* Make nutrient agar as follows:

(*a*) For the base and seed layers:

| | |
|---|---|
| Peptone | 6.0 gm. |
| Yeast extract | 3.0 gm. |
| Beef extract | 1.5 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 5.9 after sterilization.

(*b*) For carrying the test organism:

| | |
|---|---|
| Peptone | 6.0 gm. |
| Pancreatic digest of casein | 4.0 gm. |
| Yeast extract | 3.0 gm. |
| Beef extract | 1.5 gm. |
| Dextrose | 1.0 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 6.5 to 6.6 after sterilization.

(iii) *Working standard.* Dry approximately 30 milligrams of the working standard for three hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, dissolve in sufficient 0.1*N* sodium hydroxide to give a solution of 10,000 micrograms per milliliter, and then further dilute with sufficient sterile distilled water to give a stock solution of 1,000 micrograms per milliliter. This stock solution may be used for at least 1 week when stored under refrigeration (15° C. or less).

(iv) *Preparation of sample.* Dissolve an accurately weighed sample of approximately 30 milligrams in sufficient distilled water to make a stock solution of 1 milligram per milliliter. Further dilute in 0.1*M* potassium phosphate buffer, pH 4.5.

(v) *Preparation of test organism.* The test organism is *Bacillus cereus* var. *mycoides* (ATCC 11778). Maintain the test organism on the nutrient agar medium described in subdivision (ii)(*b*) of this subparagraph, and transfer to a fresh slant every month. Inoculate a fresh slant with the test organism and incubate at 30° C. for 16 hours to 24 hours. Wash the culture from the slant with sterile sodium chloride solution onto the surface of a Roux bottle containing 300 milliliters of the nutrient agar medium described in subdivision (ii)(*b*) of this subparagraph. Incubate at 30° C. for 1 week. Wash the resulting growth from the agar surface with about 30 milliliters of sterile sodium chloride solution, and heat for 30 minutes at 65° C. Wash the spore suspension three times with sterile sodium chloride solution, heat again for 30 minutes at 65° C., and resuspend in sterile sodium chloride solution. Run test plates to determine the quantity of the spore suspension to be added to each 100 milliliters of the agar described in subdivision (ii)(*a*) of this subparagraph (melted and cooled to 48° C.) for the seed layer, which will give sharp, clear zones of inhibition. This spore suspension will keep for months.

(vi) *Preparation of plates.* Add 10 milliliters of the melted agar described in subdivision (ii)(*a*) of this subparagraph to each Petri dish (20 millimeters × 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain covers glazed only on the outside, and allow to harden. Use the plates the same day they are prepared. Add 4 milliliters of the seeded agar prepared as described in subdivision (v) of this subparagraph to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface. After the seed layer has hardened, place 6 cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare concentrations for the standard curve by diluting aliquots of the standard stock solution with 0.1*M* potassium phosphate buffer, pH 4.5, to give final concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 micrograms per milliliter. Use three plates for each concentration, except the 10.0 micrograms per milliliter concentration. Thus, there will be a total of 12 plates. The 10.0-microgram concentration is the reference point and is included on each plate. On each of three plates fill three cylinders with the 10.0 micrograms per milliliter standard and the other three cylinders with the concentration of the standard under test. Thus, there will be thirty-six 10.0 micrograms per milliliter determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 30° C., and measure the diameter of each circle of inhibition. Average the readings of 10.0 micrograms per milliliter concentration and the readings of the concentration tested on each set of three plates, and average also all 36 readings of the 10.0 micrograms per milliliter concentration. The average of the 36 readings of the 10.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each concentration to the figure it would be if the 10.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 8.0 micrograms per milliliter concentration the average of the 36 readings of the 10.0 micrograms per milliliter concentration is 18 millimeters and the average of the 10.0 micrograms per milliliter concentration on this set of three plates is 17.8 millimeters, the correction is plus 0.2 millimeter. If the average reading of the 8.0 micrograms per milliliter concentration of these same three plates is 15.0 millimeters, the corrected value is then 17.2 millimeters. Plot these corrected values, including the average of the thirty-six 10.0 micrograms per milliliter concentrations, on 2-cycle semilog paper, using the concentrations in micrograms per milliliter as the ordinate (logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

$L$ = Corrected zone diameter for the lowest concentration of the standard curve;

$H$ = Corrected zone diameter for the highest concentration of the standard curve;

$c$ = Average zone diameter of 36 readings of the 10.0 milligrams per milliliter standard;

$a$, $b$, $d$, $e$ = Corrected average values for the 6.4, 8.0, 12.5, and 15.6 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for $L$ and $H$, and connect with a straight line.

(viii) *Assay procedure.* Use three plates for each sample, and fill three cylinders on each plate with the 10.0 micrograms per milliliter standard and three cylinders with the sample diluted to an estimated 10.0 micrograms per milliliter with 0.1*M* potassium phosphate buffer, pH 4.5, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 30° C. and measure the diameter of each circle of inhibition. Average the zone readings of the standard, and average the zone readings of the sample on the plates used. If the sample gives a larger average zone size than the average of the standard, add the difference between them to the 10.0 micrograms per milliliter zone size of the standard curve. If the average sample zone size is smaller than the standard value, subtract the difference between them from the 10.0 micrograms per milliliter zone size of the standard curve. From the curve, read the concentration corresponding to these corrected values of zone sizes.

(2) *Sterility.* Use the equivalent of approximately 500 milligrams (activity), and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of sterile U.S.P. saline T.S., containing 4 milligrams of vancomycin (activity) per milliliter.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1.0 milliliter per kilogram of a solution containing 10 milligrams per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 1 milliliter per kilogram of a solution containing 3 milligrams per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 50 milligrams per milliliter.

(8) *Factor A content—Chromatographic method*—(i) *Preparation of standard and sample solutions*—(a) *Vancomycin hydrochloride standard.* Dilute the vancomycin hydrochloride standard to 0.60 milligram per milliliter in distilled water.

(b) *Vancomycin A standard (Factor A).* Dilute the Factor A standard to 90.0 micrograms per milliliter in distilled water.

(c) *Vancomycin hydrochloride sample.* Dilute the vancomycin hydrochloride sample to 0.60 milligram per milliliter.

(ii) *Preparation of resolving solvent.* Prepare the resolving solvent by mixing two volumes of tertiary amyl alcohol, one volume of acetone, and two volumes of water.

(iii) *Procedure.* Using chromatographic paper described in § 141c.231(d)(5) of this chapter, cut to 8 inches x 10 inches, and draw a line of origin parallel to and 1 inch from the edge of the paper. Starting 2 inches in from the edge and at 1-inch intervals, apply 5 microliters of each of the solutions described in subdivision (i) of this subparagraph. Allow spots to dry thoroughly, and then using a descending chromatographic technique, allow the chromatogram to develop 16 hours to 18 hours at room temperature. A layer of the resolving solvent is maintained in the chamber to saturate the atmosphere. After developing time has elapsed, remove the chromatographic paper, allow to dry thoroughly, and place the chromatogram on a sheet of nutrient agar (described in subparagraph (1)(ii)(b) of this paragraph) ⅛-inch–3/16-inch thick, which has been inoculated with *Staphylococcus aureus* (ATCC 6538P) or *Bacillus subtilis* (ATCC 6633). After 30 minutes, remove the paper from the surface and incubate the inoculated agar overnight at 37° C. The resulting zones are compared visually. The sample is satisfactory if it exhibits a zone for Factor A not exceeding the zone of that of the standard Factor A used.

(9) *Identity.* Dissolve the sample at a concentration of 10 milligrams per milliliter of water.

(a) Dilute an aliquot to 1 milligram per milliliter in water.

(b) Dilute another aliquot to 1 milligram per milliliter with 0.05*N* sodium hydroxide final concentration. The ultraviolet absorption maxima is observed at 282 millimicrons ±2 millimicrons in water and at 305 millimicrons ±2 millimicrons in the alkaline solution.

(10) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

**§ 148s.2 Vancomycin hydrochloride for intravenous use.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Vancomycin hydrochloride for intravenous use is a dry mixture of vancomycin hydrochloride, with or without one or more suitable buffer substances. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 5 percent. Its pH in an aqueous solution containing 50 milligrams per milliliter is not less than 2.5 and not more than 4.5. The vancomycin hydrochloride used conforms to the requirements of § 148s.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Vancomycin hydrochloride used in making the batch (if it contains a buffer substance) for potency, Factor A content, and identity.

(*b*) The batch for potency, sterility, toxicity, pyrogens, moisture, pH, Factor A content, and identity (if it does not contain a buffer substance).

(ii) Samples required:

(*a*) Vancomycin hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: Minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container in the samples submitted in accordance with subparagraph (3)(ii)(*a*), (*b*)(*1*), and (*c*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute the sample as directed in the labeling. Using a suitable syringe, withdraw a one-dose aliquot and place in a flask. Proceed as directed in § 148s.1(b)(1). Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Use the entire contents of single-dose containers or the equivalent of approximately 500 milligrams (activity) from each multiple-dose container, and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of a solution containing 4 milligrams of vancomycin (activity) per milliliter.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution containing 10 milligrams per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 1 milliliter per kilogram of a solution containing 3 milligrams per milliliter, prepared with the diluent recommended by the manufacturer in his labeling for the drug.

(6) *Moisture.* Proceed as directed in § 141a.26(c) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 50 milligrams per milliliter.

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## PART 148t—VIOMYCIN

Sec.

148t.1 Viomycin sulfate.

148t.2 Viomycin sulfate for intramuscular injection.

AUTHORITY: §§ 148t.1 to 148t.2 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

**§ 148t.1 Viomycin sulfate.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Viomycin sulfate is the sulfate salt of viomycin. It is so purified and dried that:

(i) Its potency is not less than 720 micrograms of viomycin base per milligram, on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 3 percent.

(vii) Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) Its absorption maximum at 268 millimicrons does not vary more than ±3 millimicrons from that of the viomycin working standard, similarly treated.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, histamine, moisture, pH, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: A minimum of 10 packages, each containing 500 milligrams.

(*b*) For sterility: 10 packages, each containing 500 milligrams.

(4) *Fees.* $10.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(b) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay*—(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(1) Make nutrient agar for carrying the test organism as follows:

| | |
|---|---|
| Peptone | 6.0 gm. |
| Pancreatic digest of casein | 4.0 gm. |
| Yeast extract | 3.0 gm. |
| Beef extract | 1.5 gm. |
| Dextrose | 1.0 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 6.5 to 6.6 after sterilization.

(2) Make nutrient agar for preparing the inoculated plates as follows:

| | |
|---|---|
| Beef extract | 1.5 gm. |
| Yeast extract | 3.0 gm. |
| Peptone | 6.0 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 7.8 to 8.0 after sterilization.

(c) *Working standard.* Dry approximately 30 milligrams working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, and dissolve in sufficient distilled water to obtain a stock standard solution of 1,000 micrograms per milliliter. Keep this stock solution in a refrigerator. Do not use it later than 1 week after it is made.

(d) *Preparation of sample.* Dissolve the sample to be tested in 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration of 50 micrograms of viomycin base per milliliter (estimated).

(e) *Preparation of test organism.* The test organism is *Bacillus subtilis* (ATCC 6633). Maintain the test organism on nutrient agar prepared as described in (b)(1) of this subdivision. Prepare a spore suspension by one of the following methods:

(1) Grow the organism for 1 week at 37° C. in a number of Roux bottles, each containing 300 milliliters of nutrient agar prepared as described in (b)(1) of this subdivision. Suspend the spores in sterile distilled water and heat for 30 minutes at 65° C. Wash the spore suspension three times with sterile distilled water, heat again for 30 minutes at 65° C. and resuspend in sterile distilled water; or

(2) Grow the organism for 5 days at 37° C. in a Roux bottle containing 300 milliliters of the agar medium described in (b)(1) of this subdivision, but modified by the addition of 300 milligrams of $MnSO_4 \cdot H_2O$ per liter. Suspend the growth in 50 milliliters of sterile isotonic saline solution, centrifuge, and decant the supernatant liquid. Reconstitute the sediment and heat-shock the suspension by heating for 30 minutes at 70° C. Maintain the spore suspension at approximately 15° C. Determine by appropriate tests the quantity of spore suspension to be added to each 100 milliliters of agar for the secondary layer that will give sharp, clear zones of inhibition.

(f) *Preparation of plates.* Add 10 milliliters of melted agar prepared as described in (b)(2) of this subdivision to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates, cover with porcelain covers glazed only on the outside, and allow to harden. Use the plates the same day they are prepared. Melt the agar to be used for the second layer, cool to 55° C. to 60° C., and add the spore suspension prepared as described in (e) of this subdivision. Mix thoroughly, and add 4 milliliters to each of the plates containing the 10 milliliters of the uninoculated agar. Tilt the plates back and forth to spread the inoculated agar evenly over the surface. Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(g) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1*M* potassium phosphate buffer (pH 8.0) to obtain concentrations of 32.0, 40.0, 50.0, 62.5, and 78.1 micrograms per milliliter. Use three plates for the determination of each point on the curve, except the 50.0 micrograms per milliliter concentration, a total of 12 plates. On each of three plates fill three cylinders with the 50.0 micrograms per milliliter standard, and the other three cylinders with the concentration under test. Thus, there will be thirty-six 50-microgram determinations and nine determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 50.0 micrograms per milliliter concentration and the readings of the point tested for each set of three plates, and average also all 36 readings of the 50.0 micrograms per milliliter concentration. The average of the 36 readings of the 50.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 50.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 40.0-microgram concentration, the average of the 36 readings of the 50.0-microgram concentration were 20.0 millimeters, and the average of the 50.0-microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 40.0-microgram concentration of these same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 50.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentrations in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L = \frac{3a + 2b + c - e}{5},$$

$$H = \frac{3e + 2d + c - a}{5},$$

where:

$L$ = Corrected zone diameter for the lowest concentration of the standard curve;
$H$ = Corrected zone diameter for the highest concentration of the standard curve;
$c$ = Average zone diameter of 36 readings of the 50.0 micrograms per milliliter standard;
$a, b, d, e$ = Corrected average values for the 32.0, 40.0, 62.5, and 78.1 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for $L$ and $H$, and connect with a straight line.

(h) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 50 micrograms per milliliter solution and three cylinders with the 50.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve at 37° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 50.0 micrograms per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 50.0 micrograms per milliliter value on the curve, and read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay*—(a) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(1) Make nutrient agar for carrying the organism, as follows:

| | |
|---|---|
| Beef extract | 1.5 gm. |
| Yeast extract | 3.0 gm. |
| Peptone | 6.0 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 7.8 to 8.0 after sterilization.

(2) Make nutrient broth for preparing an inoculum of the test organism as follows:

| | |
|---|---|
| Peptone | 5.0 gm. |
| Yeast extract | 1.5 gm. |
| Beef extract | 1.5 gm. |
| Sodium chloride | 3.5 gm. |
| Glucose | 1.0 gm. |
| Dipotassium phosphate | 3.68 gm. |
| Potassium dihydrogen phosphate | 1.32 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 7.0 after sterilization.

(b) *Test culture.* Employ the agar described in (a)(1) of this subdivision for maintaining the test organism which is Klebsiella pneumoniae (ATC 10031), noncapsulated. Transfer stock cultures every week for test purposes. Transfer the organism to fresh agar slants and incubate overnight at 37° C. Suspend the growth from two or three of these slants in sterile distilled water, and add approximately 5 milliliters of culture suspension to each of two Roux bottles containing the agar described in (a)(1) of this subdivision. Incubate the bottles overnight at 37° C., harvest the growth, using 50 milliliters of sterile sodium chloride solution per bottle, and pool the washings from the two bottles. Determine the dilution with saline solution that will give a light transmission reading of 25 percent, using a suitable photoelectric colorimeter with a 580-millimicron filter and an 18-millimeter test tube as an absorption cell. Keep the resulting suspension of organisms in the refrigerator and use for a period not to exceed 2 weeks. Prepare the daily inoculum by adding 0.1 milliliter–0.2 milliliter of the suspension to each 100 milliliters of the nutrient broth prepared as directed in (a)(2) of this subdivision, cooled to a temperature of approximately 15° C.

(c) *Working standard and standard curve.* To prepare solutions for the standard curve, dilute the 1,000 micrograms per milliliter working standard described in subdivision (i) (c) of this subparagraph in distilled water to make concentrations of 64, 80, 100, 125, and 156 micrograms per milliliter. The 100 micrograms per milliliter concentration is the reference point of the curve. Add 1.0 milliliter of each dilution to each of three test tubes (16 millimeters x 125 millimeters).

(d) *Preparation of sample.* Dilute the sample under test in sterile distilled water to contain 100 micrograms per milliliter (estimated). Add 1.0 milliliter of this dilution to each of three test tubes (16 millimeters x 125 millimeters).

(e) *Procedure.* To each of the tubes prepared as described in (c) and (d) of this subdivision, add 9.0 milliliters of the inoculated broth prepared as outlined in (b) of this subdivision. Place all tubes immediately in a water bath at 37° C. for 3 hours to 4 hours. After incubation, add 0.5 milliliter of 12 percent formaldehyde to each tube. Read the absorbance values of each tube in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument to zero absorbance with clear, uninoculated broth.

(f) *Estimation of potency.* Plot the average absorbance values for each concentration of the standard on two-cycle semilogarithmic graph paper with absorbance values on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight line through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

$L$=Absorbance value for the lowest concentration of the standard curve;

$H$=Absorbance value for the highest concentration of the standard curve;

$a, b, c, d, e$=Average absorbance values for each concentration of the standard curve.

Plot the values obtained for $L$ and $H$, and connect the points with a straight line. Average the absorbance value for the sample and read the viomycin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the viomycin content of the sample.

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Using as a test dose, 1.0 milliliter per kilogram of a sterile, pyrogen-free U.S.P. saline test solution containing 10 milligrams per milliliter, proceed as directed in § 141a.3 of this chapter.

(4) *Toxicity.* Using as a test dose 0.5 milliliter of a sterile U.S.P. saline test solution containing 10 milligrams per milliliter, proceed as directed in § 141a.4 of this chapter.

(5) *Histamine.* Using as a test dose 0.6 milliliter per kilogram of a sterile U.S.P. saline test solution containing 5 milligrams per milliliter, proceed as directed in § 141b.105 of this chapter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 100 milligrams of viomycin base per milliliter.

(8) *Identity*—(i) *Phosphate buffer solution.* Mix 296.3 milliliters of 0.1*N* sodium hydroxide with 500 milliliters of 0.05*M* postassium phosphate (6.805 grams of $KH_2PO_4$ per 1,000 milliliters of water) and dilute with sufficient water to make 1 liter.

(ii) *Procedure.* Dissolve the working standard and the sample to be tested in sufficient potassium phosphate buffer solution to give solutions containing 10 micrograms per milliliter. Measure the ultraviolet absorption of each solution in a suitable spectrophotometer. Compare the spectrum of the sample qualitatively with that of the standard.

**§ 148t.2 Viomycin sulfate for intramuscular injection.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Viomycin sulfate for intramusular injection is a dry mixture of viomycin sulfate, with or without one or more suitable buffer substances. It is sterile. It is nonpyrogenic. It is nontoxic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 3 percent. Its pH in an aqueous solution containing 100 milligrams per milliliter is not less than 4.5 and not more than 7.0. The viomycin sulfate used conforms to the requirements of § 148t.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefore by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Viomycin sulfate used in making the batch for potency and identity.

(*b*) The batch for potency, sterility, toxicity, pyrogens, histamine, moisture, and pH.

(ii) Samples required:

(*a*) Viomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container in the samples submitted in accordance with subparagraph (3) (ii) (*a*), (*b*) (*1*), and (*c*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3) (ii) (*b*) (*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute the sample as directed in the labeling. Using a suitable syringe, withdraw a one-dose aliquot and place in a flask. Proceed as directed in § 148t.1(b)(1). Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Use the entire contents of single-dose containers or the equivalent of approximately 500 milligrams (activity) from each multiple-dose container, and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of a sterile U.S.P. saline test solution containing 2 milligrams per milliliter.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a sterile, pyrogen-free U.S.P. saline test solution containing 10 milligrams per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter per kilogram of a sterile U.S.P. saline test solution containing 5 milligrams per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 100 milligrams per milliliter.

All interested persons are invited to present their views in writing regarding this proposal to the Hearing Clerk, Department of Health, Education, and Welfare, Washington 25, D.C. within 30 days from the date of publication of this notice in the FEDERAL REGISTER. Such views and comments should be filed preferably in quintuplicate.

Proposals for the certification of various other antibiotic drugs will be published in the immediate future.

Dated: April 9, 1963.

GEO. P. LARRICK,
*Commissioner of Food and Drugs.*

[F.R. Doc. 63-3985; Filed, Apr. 17, 1963; 8:45 a.m.]

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## [ 21 CFR Part 148n ]

## OXYTETRACYCLINE

### Proposed Regulations for Tests and Methods of Assay and Certification of Oxytetracycline and Oxytetracycline-Containing Drugs

The Kefauver-Harris Drug Amendments of 1962 provide that all antibiotic drugs intended for human use shall be subject to the certification provisions of sections 502(1) and 507 of the Federal Food, Drug, and Cosmetic Act. These amendments become effective May 1, 1963. Therefore, the Commissioner of Food and Drugs, pursuant to the provisions of the act (sec. 507, 59 Stat. 463,

as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (25 F.R. 8625), proposes to issue the following new regulation to provide for tests and methods of assay and certification of oxytetracycline:

## PART 148n—OXYTETRACYCLINE

Sec.


148n.1 Oxytetracycline.
148n.2 Oxytetracycline hydrochloride.
148n.3 Calcium di-oxytetracycline.
148n.4 Oxytetracycline capsules; oxytetracycline hydrochloride capsules.
148n.5 Oxytetracycline intramuscular solution.
148n.6 Oxytetracycline tablets.
148n.7 Oxytetracycline troches.
148n.8 Oxytetracycline for oral suspension.
148n.9 Oxytetracycline-nyastatin for oral suspension.
148n.10 Oxytetracycline-nystatin capsules.
148n.11 Oxytetracycline hydrochloride for injection.
148n.12 Oxytetracycline hydrochloride soluble tablets.
148n.13 Oxytetracycline hydrochloride dental cones.
148n.14 Oxytetracycline hydrochloride dental paste.
148n.15 [Reserved]
148n.16 Oxytetracycline hydrochloride for nasal solution.
148n.17 Oxytetracycline hydrochloride for ophthalmic solution.
148n.18 Oxytetracycline hydrochloride ophthalmic ointment.
148n.19 Oxytetracycline hydrochloride topical ointment.
148n.20 Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment.
148n.21 Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment.
148n.22 Oxytetracycline hydrochloride ophthalmic oil suspension.
148n.23 Oxytetracycline hydrochloride eye and ear suspension.
148n.24 Calcium di-oxytetracycline syrup.


AUTHORITY: §§ 148n.1 to 148n.24 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

### § 148n.1 Oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline is the hydrated or anhydrous crystalline compound of a kind of oxytetracycline, or a mixture of two or more such compounds. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substance.

(vi) Its moisture content is not more than 7.5 percent.

(vii) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) When calculated on the anhydrous basis, its absorptivity at 353 millimicrons is 100±4 percent of the oxytetracycline standard similarly treated.

(ix) It gives a positive result to an identity test for oxytetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, absorptivity, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately equal portions of not less than 0.5 gram.

(*b*) For sterility testing: 10 packages, each containing approximately 40 milligrams.

(4) *Fees.* $10.00 for each package in the sample submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all packages in the sample submitted in accordance with paragraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Plate assay*—(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*1*) Make nutrient agar for maintaining the test organism as follows:

| | |
|---|---|
| Peptone | 6.0 gm. |
| Pancreatic digest of casein | 4.0 gm. |
| Yeast extract | 3.0 gm. |
| Beef extract | 1.5 gm. |
| Dextrose | 1.0 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 6.5 to 6.6 after sterilization.

(*2*) Make nutrient agar for the seed layer and for the base layer as follows:

| | |
|---|---|
| Peptone | 6.0 gm. |
| Yeast extract | 3.0 gm. |
| Beef extract | 1.5 gm. |
| Agar | 15.0 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 5.8 to 6.0 after sterilization.

In lieu of preparing the media from the individual ingredients specified in this subsection, the media may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the individual ingredients specified are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(*c*) *Working standard.* Accurately weigh a suitable quantity of the oxytetracycline working standard and dissolve in 0.1*N* hydrochloric acid to make an appropriate stock solution. Keep in a glass-stoppered flask and store in the refrigerator for not more than 3 days.

(*d*) *Preparation of sample.* Dissolve the sample to be tested in 0.1*N* hydrochloric acid to make an appropriate stock solution. Make the final dilution in 0.1*M* potassium phosphate buffer, pH 4.5, to contain 1.0 microgram per milliliter (estimated).

(*e*) *Preparation of suspension.* The test organism is *Bacillus cereus* var. *mycoides* (ATCC 11778), which is maintained on slants of agar described in subdivision (i)(*b*)(*1*) of this subparagraph. Wash the organism from the agar slant with 3 milliliters of sterile sodium chloride solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in subdivision (i)(*b*)(*1*) of this subparagraph. Spread the suspension of organism over the entire agar surface with the aid of sterile glass beads. Incubate for 1 week at 37° C. Using 50 milliliters of sterile sodium chloride solution, wash the growth from the agar surface into a glass-stoppered Erlenmeyer flask. Heat-shock this suspension for 30 minutes at 65° C. Wash three times with sterile sodium chloride solution, centrifuging between each washing, and then heat-shock for 30 minutes at 65° C. Resuspend in 50 milliliters of sterile sodium chloride solution and maintain in a refrigerator. This spore suspension may be kept for 1 month. Run test plates to determine the quantity of spore suspension (usually 0.1 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(*f*) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (i)(*b*)(*2*) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates, and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in subdivision (i)(*b*)(*2*) of this subparagraph, cool to 48° C., add the proper amount of the test organism as described in subdivision (i)(*d*) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. Place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Prepare the daily standard curve by further diluting the stock working standard solution in 0.1*M* potassium phosphate buffer, pH 4.5, to obtain concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter. Use three plates for the determination of each point on the curve, except the 1.0 microgram per milliliter concentration, a total of 12 plates. On each of three plates fill three cylinders with the 1.0 micrograms per milliliter standard, and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1-microgram determinations and 9 determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition of the plates. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8 microgram per milliliter concentration, the average of the 36 readings of the 1.0 microgram

concentration were 20.0 millimeters, and the average of the 1.0 microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 0.8-microgram concentration of these same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

$L$ = Corrected zone diameter for the lowest concentration of the standard curve;
$H$ = Corrected zone diameter for the highest concentration of the standard curve;
$c$ = Average zone diameter of 36 readings of the 1.0 microgram per milliliter standard;
$a, b, d, e$ = Corrected average values for the 0.64, 0.80, 1.25, and 1.56 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for $L$ and $H$ and connect with a straight line.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 1.0 microgram per milliliter solution and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 30° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, substract the difference between them from the 1.0 microgram per milliliter value on the curve. From the standard curve, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay.* In lieu of the plate assay method described in subdivision (i) (*h*) of this subparagraph, the sample may be assayed for potency by the following turbidimetric method:

(*a*) *Nutrient broth:* Make nutrient broth for preparing an inoculum of the test organism as follows:

| | |
|---|---|
| Peptone | 5.0 gm. |
| Yeast extract | 1.5 gm. |
| Beef extract | 1.5 gm. |
| Sodium chloride | 3.5 gm. |
| Dextrose | 1.0 gm. |
| Dipotassium phosphate | 3.68 gm. |
| Potassium dihydrogen phosphate | 1.32 gm. |
| Distilled water, q.s. | 1,000.0 ml. |

pH 7.0 after sterilization.

In lieu of preparing the medium from the individual ingredients specified in this subsection, the medium may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the individual ingredients specified are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(*b*) *Working standard.* Accurately weigh a suitable quantity of the oxytetracycline working standard and dissolve in 0.1*N* hydrochloric acid to make an appropriate stock solution. Keep in a glass-stoppered flask and store in the refrigerator for not more than 1 week.

(*c*) *Preparation of sample.* Dissolve an appropriate quantity of the sample in a volume of 0.1*N* hydrochloric acid to obtain a convenient stock solution. Make other dilutions volumetrically in 0.1*M* potassium phosphate buffer, pH 4.5, to a final concentration of 0.24 microgram of oxytetracycline base per milliliter (estimated).

(*d*) *Preparation of test organism.* The test organism is *Staphylococcus aureus* (ATCC 6538P), which is maintained on agar described in subdivision (i) (*b*) (*1*) of this subparagraph. From a stock slant, inoculate a Roux bottle containing the same agar and incubate for 24 hours at 37° C. Wash the growth from the agar surface with 50 milliliters of sterile sodium chloride solution. Standardize the resulting bulk suspension so that a 1:20 dilution in sodium chloride solution will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. For the daily inoculum, use approximately 3.0 milliliters of the bulk suspension for each 1,000 milliliters of nutrient broth needed in the assay.

(*e*) *Procedure.* Prepare solutions for the daily standard curve by diluting an aliquot of the stock working standard solution prepared as described in subdivision (ii) (*b*) of this subparagraph in 0.1*M* potassium phosphate buffer, pH 4.5, to the following concentrations: 0.146, 0.187, 0.240, 0.308, and 0.395 microgram per milliliter. Place 1.0 milliliter of each concentration of the standard curve and of the sample solution prepared as described in subdivision (ii) (*c*) of this subparagraph in each of three replicate test tubes (16 millimeters x 125 millimeters). To each tube add 9 milliliters of the inoculated broth described in subdivision (ii) (*d*) of this subparagraph, and place immediately in a water bath at 37° C. for 3 to 4 hours. Remove the tubes and add 0.5 milliliter of a 12 percent solution of formaldehyde to each tube. Read the absorbance values of each tube in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear, uninoculated broth prepared as described in subdivision (ii) (*a*) of this subparagraph.

(*f*) *Estimation of potency.* Plot the average absorbance values for each concentration of the standard on two-cycle semilogarithmic graph paper with absorbance values on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight line through the points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

$L$ = Absorbance value for the lowest concentration of the standard curve;
$H$ = Absorbance value for the highest concentration of the standard curve;
$a, b, c, d, e$ = Average absorbance values for each concentration of the standard curve.

Plot the values obtained for $L$ and $H$ and connect the points with a straight line. Average the absorbance value for the sample and read the oxytetracycline concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the oxytetracycline content of the sample.

(iii) *Chemical assay.* In lieu of the biological assay methods described in this paragraph, the sample may be assayed for potency by the following chemical assay method:

(*a*) *Reagents.* (*1*) 1*N* hydrochloric acid.

(*2*) 0.01*N* hydrochloric acid.

(*3*) Ferric chloride stock solution.

Quickly weigh (very hygroscopic) 5.0 grams of $FeCl_3 \cdot 6H_2O$ into a 100-milliliter beaker. Add approximately 10 milliliters of 1*N* hydrochloric acid, and stir to dissolve. Quantitatively transfer to a 50-milliliter, glass-stoppered, amber volumetric flask, and make up to volume with water.

(*4*) Ferric chloride working reagent.

Pipette 10.0 milliliters of ferric chloride stock solution to a 1-liter volumetric flask, add 10.0 milliliters 1*N* hydrochloric acid and bring to volume with water. Check the pH; it should be between 2.0 and 2.1.

(*b*) *Standard solution.* Accurately weigh 50 milligrams of the oxytetracycline working standard, and add sufficient 0.1*N* hydrochloric acid to obtain a solution containing 2,000 micrograms per milliliter. Swirl to dissolve the standard, and add sufficient distilled water to give a solution containing exactly 200 micrograms of oxytetracycline per milliliter. This solution may be used for 1 week if stored in the refrigerator.

(*c*) *Sample solution.* Accurately weigh 50 milligrams of sample into a 250-milliliter volumetric flask. Add 2.50 milliliters of 0.1*N* hydrochloric acid. Swirl to dissolve the sample, and make up to volume with water.

(*d*) *Procedure.* Pipette exactly 10.0 milliliters of the standard solution and of the sample solution into separate test tubes. To each tube add exactly 10.0 milliliters of ferric chloride working reagent, mix and allow to stand 15 minutes. Determine the absorbance of each solution at 490 millimicrons in a suitable spectrophotometer against a blank prepared from 10 milliliters of 0.01*N* hydrochloric acid and 10 milliliters of ferric chloride working reagent.

(*e*) *Estimation of potency.* Calculate the potency as follows:

$$\text{Micrograms of oxytetracycline per milligram} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \frac{\text{Potency of standard in micrograms per milligram}}{\text{Total dilution of standard in milliliters}} \times 250.$$

(2) *Sterility.* Using 40 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of an aqueous solution containing 2.0 milligrams per milliliter prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile distilled water.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of an aqueous solution containing 500 micrograms per milliliter, prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of pyrogen-free, sterile distilled water.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter per kilogram of a sterile distilled water solution containing 5 milligrams per milliliter, prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile distilled water.

(6) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 141 a.5(b) of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(8) *Absorptivity.* Dissolve approximately 50 milligrams of the sample in 250 milliliters of 0.01*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer, determine the absorbance of the solution at 353 millimicrons. Run a standard simultaneously. Calculate absorptivity values for standard and sample.

$$\text{Absorptivity of sample} = \frac{\text{Absorbance at 353 millimicrons} \times 25{,}000}{\text{Weight of sample in milligrams} \times (100 - M)}.$$

where:

$M$ = Percent moisture in the sample.

$$\text{Absorptivity of standard} = \frac{\text{Absorbance at 353 millimicrons} \times 25{,}000{,}000}{\text{Weight of standard in milligrams} \times \text{Potency of standard in micrograms per milligram}}.$$

$$\text{Absorptivity} = \frac{\text{Absorptivity of sample} \times 100}{\text{Absorptivity of standard}}.$$

(9) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(10) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

### § 148n.2 Oxytetracycline hydrochloride.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride is the crystalline hydrochloride salt of a kind of oxytetracycline or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 835 micrograms per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substance.

(vi) Its moisture content is not more than 1.5 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0.

(viii) When calculated on the anhydrous basis, its absorptivity at 353 millimicrons is 92.5±4.3 percent of the oxytetracycline working standard similarly treated.

(ix) It gives a positive result to an identity test for oxytetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, absorptivity, crystallinity, and identity.

(ii) Samples of the batch:

(*a*) For all tests except sterility: 10 packages, each containing approximately equal portions of not less than 0.5 gram.

(*b*) For sterility testing: 10 packages, each containing approximately 40 milligrams.

(4) *Fees.* $10.00 for each package in the sample submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all packages in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148n.1(b)(1).

(2) *Sterility.* Proceed as directed in § 148n.1(b)(2).

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of a solution in sterile distilled water containing 2.0 milligrams (activity) per milliliter.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution in sterile, pyrogen-free distilled water containing 500 micrograms per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter per kilogram of a sodium chloride solution containing 5 milligrams per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Absorptivity.* Proceed as directed in § 148n.1(b)(8).

(9) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(10) *Identity.* Proceed as directed in § 148n.1(b)(10).

### § 148n.3 Calcium di-oxytetracycline.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Calcium di-oxytetracycline is the crystalline calcium salt of a kind of oxytetracycline or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is equivalent to not less than 865 micrograms of oxytetracycline base per milligram on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 14 percent.

(iv) Its pH in a saturated aqueous suspension containing 25 milligrams per milliliter is not less than 6.0 and not more than 8.0.

(v) Its calcium content is not less than 4 percent and not more than 4.5 percent on an anhydrous basis.

(vi) It gives a positive result to the identity test for oxytetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, calcium content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately equal portions of not less than 0.5 gram.

(4) *Fees.* $4.00 for each package in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148n.1(b)(1).

(2) *Toxicity.* Proceed as directed in § 148n.1(b)(3).

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a saturated aqueous suspension containing 25 milligrams per milliliter.

(5) *Calcium content.* Place approximately 1 gram of the sample, accurately weighed, in a tared porcelain crucible, and carefully ignite at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain

lid during the charring. Add to the contents of the crucible 2.0 milliliters of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° C. to 600° C. until the carbon is all burned off. Cool the crucible in a desiccator and weigh. From the weight of residue obtained, calculate the calcium content as follows:

$$\text{Percent calcium} = \frac{\text{Weight of residue} \times 0.29435}{\text{Weight of sample}} \times 100.$$

(6) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(7) *Identity.* Proceed as directed in § 148n.1(b)(10).

**§ 148n.4 Oxytetracycline capsules; oxytetracycline hydrochloride capsules.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline capsules and oxytetracycline hydrochloride capsules are capsules composed of crystalline oxytetracycline or crystalline oxytetracycline hydrochloride, with or without one or more suitable sulfonamides, and with or without one or more suitable and harmless vitamin substances, vegetable oils, buffers, preservatives, diluents, binders, lubricants, colorings, and flavorings, enclosed in a gelatin capsule. The potency of each capsule is not less than 50 milligrams. The moisture content is not more than 5.0 percent if it contains oxytetracycline hydrochloride and not more than 7.5 percent if it contains oxytetracycline, except that in no case shall it be more than 3.0 percent if it contains vitamins. The oxytetracycline used conforms to the requirements of § 148n.1(a)(1)(i), (iii), (vi), (vii), (viii), and (ix). The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a)(1)(i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of §148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline or oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Oxytetracycline or oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: Minimum 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Using a representative number of capsules (usually 5 to 12) and 500 milliliters of 0.1*N* hydrochloric acid in a blender, proceed as directed in § 148n.1(b)(1). The potency of the drug is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* If it contains oxytetracycline hydrochloride, proceed as directed in § 141a.5(a) of this chapter, and if it contains oxytetracycline proceed as directed in § 141a.26(e) of this chapter.

**§ 148n.5 Oxytetracycline intramuscular solution.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline intramuscular solution is oxytetracycline, with or without one or more suitable and harmless buffer substances, dissolved in one or more suitable and harmless solvents. Its potency is not less than 50 milligrams per milliliter. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its pH is not less than 8.3 and not more than 8.8. The oxytetracycline used conforms to the requirements of § 148n.1(a)(1). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, toxicity, pyrogens, histamine, and pH.

(ii) Samples required:

(*a*) Oxytetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 8 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $10.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $4.00 for each container in the samples submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $10.00 for all containers in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1)(i) or (ii), except prepare the sample as follows: Transfer a representative quantity of the sample equivalent to one dose to a 100-milliliter volumetric flask. Make to mark with 0.1*N* hydrochloric acid. Further dilute in pH 4.5 buffer solution to the prescribed reference concentration. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams per milliliter that it is represented to contain.

(2) *Sterility.* Use the entire contents of single-dose containers or the equivalent of approximately 40 milligrams (activity) from each multiple-dose container, and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except use sterile distilled water as the diluent, and inject 0.5 milliliter of a solution containing 2 milligrams per milliliter.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution containing 500 micrograms of oxytetracycline per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter per kilogram of a solution containing 5 milligrams per milliliter.

(6) *pH.* Using the undiluted solution, proceed as directed in §141a.5(b) of this chapter.

**§ 148n.6 Oxytetracycline tablets.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline tablets are tablets composed of oxytetracycline, with or without one or more suitable and harmless diluents, lubricants, colorings, and flavorings. The potency of each tablet is not less than 250 milligrams. The moisture content is not more than 7.5 percent. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The oxytetracycline used conforms to the requirements of § 148n.1(a)(1)(i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration time: Six tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each tablet in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b)(1) (i) or (ii), except prepare the sample as follows: Place a representative number of tablets (usually 3 to 6) in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend for 3 minutes and then make the proper estimated dilutions in 0.1*M* potassium phosphate buffer, pH 4.5, to the prescribed reference concentration. The average potency of the tablets is satisfactory if they contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

### § 148n.7 Oxytetracycline troches.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline troches are troches composed of oxytetracycline and one or more suitable and harmless diluents, binders, and lubricants, with or without one or more suitable and harmless preservatives, colorings, and flavorings. The potency of each troche is not less than 15 milligrams. The moisture content is not more than 4 percent. The oxytetracycline used conforms to the requirements of § 148n.1(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 troches.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each troche in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b)(1) (i) or (ii), except prepare the sample as follows: Place a representative number of troches in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend for 3 to 5 minutes and then make the proper estimated dilutions in pH 4.5 buffer solution to the prescribed reference concentration. The average potency of the troches is satisfactory if they contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

### § 148n.8 Oxytetracycline for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline for oral suspension is oxytetracycline, with or without glucosamine hydrochloride, and with or without suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When prepared as directed in the labeling, each milliliter contains not less than 50 milligrams of oxytetracycline. Its moisture content is not more than 2 percent. The oxytetracycline used conforms to the requirements of § 148n.1(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F. conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Oxytetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each container in the samples submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1) (i) or (ii), except prepare the sample as follows: Reconstitute as directed in the labeling. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a 100-milliliter volumetric flask, and make to mark with 0.1*N* hydrochloric acid. Withdraw an aliquot from the volumetric flask, and using pH 4.5 buffer solution dilute to the prescribed reference concentration. Its content of oxytetracycline is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

### § 148n.9 Oxytetracycline-nystatin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline-nystatin for oral suspension is oxytetracycline and nystatin, with or without glucosamine hydrochloride, and with or without suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter shall contain not less than 25 milligrams of oxytetracycline and not less than 25,000 units of nystatin. Its moisture content is not more than 2 percent. The oxytetracycline used conforms to the requirements of § 148n.1(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). The nystatin used conforms to the requirements of § 148k.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The batch for oxytetracycline content, nystatin content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The nystatin used in making the batch: 10 packages, each containing 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $5.00 for each immediate container in the sample submitted in ac-

cordance with subparagraph (3)(ii)(c) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(a), (b), and (d) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 148n.1(b)(1)(i) or (ii), except prepare the sample as follows: Reconstitute the powder as directed in the labeling of the drug. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a 100-milliliter volumetric flash, and make to mark with 0.1*N* hydrochloric acid. Withdraw an aliquot from the volumetric flash and, using pH 4.5 buffer, dilute to the prescribed reference concentration. Its content of oxytetracycline is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Nystatin content.* Proceed as directed in § 148k.1(b)(1) of this chapter, except prepare the sample as follows: Reconstitute the powder as directed in the labeling of the drug. Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a blending jar containing 150 milliliters of dimethyl formamide. Blend for 2 minutes in a high-speed blender and then dilute an aliquot with sufficient dimethyl formamide to give a nystatin concentration of 400 units per milliliter (estimated) if the plate assay method is used, or 160 units per milliliter (estimated) if the turbidimetric assay method is used. Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter (estimated) if the plate assay method is used, or with distilled water to 16 units per milliliter (estimated) if the turbidimetric assay method is used. Its content of nystatin is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of units it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

**§ 148n.10 Oxytetracycline-nystatin capsules.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline-nystatin, capsules are capsules composed of oxytetracycline and nystatin with or without one or more suitable and harmless buffer substances, vegetable oils, preservatives, diluents, binders, lubricants, colorings, flavorings, and glucosamine hydrochloride, enclosed in a gelatin capsule. Each capsule contains not less than 250 milligrams of oxytetracycline and not less than 250,000 units of nystatin. Its moisture content is not more than 2 percent. The oxytetracycline used conforms to the requirements of § 148n.1(a)(1)(i), (iii), (vi), (vii), (viii), and (ix). The nystatin used conforms to the requirements of § 148k.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(b) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(c) The batch for oxytetracycline content, nystatin content, and moisture.

(ii) Samples required:

(a) The oxytetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) Nystatin used in making the batch: 10 packages, each containing 500 milligrams.

(c) The batch: A minimum of 30 capsules.

(d) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $1.00 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(c) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(a), (b), and (d) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 148n.1(b)(1)(i) or (ii), except prepare the sample as follows: Place a representative number of capsules in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend for 3 to 5 minutes, and then make the proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. Its potency is satisfactory if it contains the equivalent of not less than 85 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain.

(ii) *Nystatin content.* Proceed as directed in § 148k.1(b)(1) of this chapter, except prepare the sample as follows: Place a representative number of capsules in a blending jar with sufficient dimethyl formamide to give a stock solution of convenient concentration. Blend for 2 minutes in a high-speed blender. Dilute an aliquot of the blended solution in sufficient dimethyl formamide to give a nystatin concentration of 400 units per milliliter (estimated) if the plate assay method is used, or 160 units per milliliter (estimated) if the turbidimetric assay method is used. Further dilute with 10 percent potassium phosphate buffer, pH 6.0, to 20 units per milliliter (estimated) if the plate assay method is used, or with distilled water to 16 units per milliliter (estimated) if the turbidimetric assay method is used. Its content of nystatin is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of units it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

**§ 148n.11 Oxytetracycline hydrochloride for injection.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride for injection is a dry mixture of oxytetracycline hydrochloride with a suitable buffer, the latter usually consisting of an amino acid. Each vial contains the equivalent of not less than 100 milligrams of oxytetracycline base. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 2.5 percent. The pH of an aqueous solution containing 10 milligrams of oxytetracycline base per milliliter is not less than 8.5 and not more than 9.5. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays:

(a) The oxytetracycline hydrochloride used in making the batch for potency, moisture, absorptivity, crystallinity, and identity.

(b) The batch for potency, sterility, toxicity, pyrogens, histamine, moisture, and pH.

(ii) Samples required:

(a) Oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 10 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $10.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii)(b)(1) of this paragraph; $10.00 for all immediate containers in the sample submitted in accordance with subparagraph (3)(ii)(b)(2) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(a) and (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1)(i) or (ii), except prepare the sample as follows: Prepare an appropriate stock solution of the sample in 0.1*N* hydrochloric acid. Dilute further in pH 4.5 buffer to the prescribed reference concentration. The average potency is satisfactory if the vials contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Sterility.* Using 40 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of sterile distilled water solution containing 2.0 milligrams per milliliter.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a sterile, pyrogen-free, distilled water solution containing 5.0 milligrams per milliliter.

(4) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter per kilogram of a solution containing 5 milligrams per milliliter prepared with the diluent recommended by the manufacturer in the labeling for the drug.

(5) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

**§ 148n.12 Oxytetracycline hydrochloride soluble tablets.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride soluble tablets are composed of oxytetracycline hydrochloride with one or more suitable and harmless diluents, binders, and lubricants. Each tablet contains the equivalent of not less than 50 milligrams of oxytetracycline base. The moisture content is not more than 1.5 percent. The oxytetracycline hydrochloride used conforms with the requirements of § 148n.2(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each tablet in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1) (i) or (ii), except prepare the sample as follows: Place a representative number of tablets in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend for 3 minutes and then make the proper estimated dilutions in 0.1*M* potassium phosphate buffer, pH 4.5. The average potency of the tablet is satisfactory if they contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5 of this chapter.

**§ 148n.13 Oxytetracycline hydrochloride dental cones.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride dental cones are dental cones composed of oxytetracycline hydrochloride and one or more suitable and harmless diluents, binders, and lubricants, with or without one or more suitable and harmless preservatives, colorings, flavorings, and local anesthetics. The potency of each dental cone is equivalent to not less than 5 milligrams of oxytetracycline base. The moisture content is not more than 2 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 dental cones.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each dental cone in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraphs (3)(ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1) (i) or (ii), except prepare the sample as follows: Place a representative number of dental cones in a glass blending jar containing 500 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend 3 to 5 minutes and then make the proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer solution. The average potency of the dental cones is satisfactory if they contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

**§ 148n.14 Oxytetracycline hydrochloride dental paste.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride dental paste is oxytetracycline hydrochloride in a suitable base, with or without one or more suitable and harmless binders, colorings, and flavorings. Its potency is equivalent to not less than 30 milligrams oxytetracycline base per gram. Its moisture content is not more than 3.0 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Oxytetracycline hydrochloride used in making the batch: 10 packages each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1) (i) or (ii), except prepare the sample as follows: Place a representative portion of the sample (usually 2.0 grams, accurately weighed) in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. The potency is satisfactory if it contains

not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.3(b) of this chapter.

**§ 148n.15 Oxytetracycline hydrochloride for inhalation.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride for inhalation is dry oxytetracycline hydrochloride packaged in combination with a suitable diluent. Its moisture content is not more than 1.5 percent. When reconstituted as directed in the labeling, each milliliter contains not less than 50 milligrams of oxytetracycline base. Its pH is not less than 2.0 and not more than 3.0. The oxytetracycline hydrochloride used in making the batch conforms to the requirements of § 148n.2(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) Oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1) (i) or (ii), except prepare the sample as follows: Reconstitute the sample as directed in the labeling. Dilute a representative aliquot in 0.1*N* hydrochloric acid to give a convenient stock solution. Dilute further in pH 4.5 buffer to the prescribed reference concentration. The average potency is satisfactory if the vials contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the sample reconstituted as directed in the labeling.

**§ 148n.16 Oxytetracycline hydrochloride for nasal solution.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride for nasal solution is a packaged combination of one immediate container of oxytetracycline hydrochloride with a suitable vasoconstrictor, with or without suitable and harmless diluents, preservative, coloring, and flavorings, and one immediate container of a suitable solvent. The oxytetracycline is of such quantity that when dissolved as directed in the labeling the potency of such solution is equivalent to not less than 5 milligrams oxytetracycline base per milliliter and maintains its labeled potency after it has been kept for 2 days in the refrigerator. Such a solution has a pH of not less than 7.8 and not more than 8.5. The moisture content of the dry mixture is not more than 5.0 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefore by such official compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3 of this chapter, each package shall bear on the outside wrapper or container and on the immediate container of the solution in the packaged combination a statement giving the method of dissolving the oxytetracycline hydrochloride and the conditions under which the solution should be stored, including a reference to its instability when stored under other conditions, and the statement "The solution may be kept in a refrigerator for 2 days without significant loss of potency." The expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, moisture, pH.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1(b)(1) (i) or (ii), except prepare the sample as follows: Reconstitute the sample as directed on the label or in the labeling. Withdraw a 1-milliliter aliquot and place in a 100-milliliter volumetric flask. Make to mark with 0.1*N* hydrochloric acid. Further dilute with pH 4.5 buffer to the prescribed reference concentration. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *pH.* Using the suspension prepared as directed in the labeling, proceed as directed in § 141a.5(b) of this chapter.

**§ 148n.17 Oxytetracycline hydrochloride for ophthalmic solution.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride for ophthalmic solution is oxytetracycline hydrochloride, with or without one or more suitable and harmless preservatives, buffer substances, and diluents. It is sterile. Its moisture content is not more than 5.0 percent. The oxytetracycline hydrochloride is of such quantity that when dissolved as directed in the labeling the potency of such solution is equivalent to not less than 5.0 milligrams oxytetracycline base per milliliter, and the solution maintains its labeled potency after it has been kept for 2 days in the refrigerator. Such solution has a pH of not less than 7.0 and not more than 8.5. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a)(1) (i), (iii), (vi), (vii), (viii), and (ix). Each other preservative, buffer, and diluent used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3 of this chapter, each package shall bear on the outside wrapper or container and on the immediate container of the solution in the packaged combination, a statement giving the method of dissolving the oxytetracycline hydrochloride and the conditions under which the solution should be stored, including reference to its instability when stored under other conditions, and the statement "The solution may be kept in a refrigerator for 2 days without significant loss of potency." The expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, toxicity, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, moisture, and pH.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3) (ii) (a), (b) (1), and (c) of this paragraph; $10.00 for all immediate containers in the samples submitted in accordance with subparagraph (3) (ii) (b) (2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b) (1) (i) or (ii), except prepare the sample as follows: Reconstitute the sample as directed in the labeling. Dilute a representative aliquot in 0.1*N* hydrochloric acid to give a convenient stock solution. Dilute further in pH 4.5 buffer to the prescribed reference concentration. The average potency is satisfactory if the vials contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Sterility.* Using the entire contents from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Moisture.* Proceed as directed in § 141a.5 (a) of this chapter.

(4) *pH.* Proceed as directed in § 141 a.5 (b) of this chapter, using a solution prepared as directed in the labeling of the drug.

**§ 148n.18 Oxytetracycline hydrochloride ophthalmic ointment.**

(a) *Requirements for certification*—(1) *Standards of indentity, strength, quality, and purity.* Oxytetracycline hydrochloride ophthalmic ointment is oxytetracycline hydrochloride in a suitable and harmless ointment base. Its potency is equivalent to not less than 10 milligrams of oxytetracycline base per gram. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2 (a) (1) (i), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(b) The batch: A minimum of 5 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b) (1) (i) or (ii), except prepare the sample as follows: Place a representative portion of the sample (usually approximately 1.0 gram, accurately weighed) in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake ointment and ether until homogeneous. Shake with a 25-milliliter portion of 0.1*N* hydrochloric acid. Remove the acid layer and repeat the extraction with three 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives, and make the proper estimated dilutions in the prescribed reference concentration in pH 4.5 buffer. The sample may also be prepared by placing a representative portion of the sample (usually 1.0 gram, accurately weighed) in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8 (b) of this chapter.

**§ 148n.19 Oxytetracycline hydrochloride topical ointment.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride topical ointment is oxytetracycline hydrochloride in a suitable and harmless ointment base. It contains cortisone, hydrocortisone, or a suitable ester of cortisone or hydrocortisone and one or more suitable and harmless preservatives and stabilizing agents. Its potency is equivalent to not less than 30 milligrams of oxytetracycline base per gram. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2 (a) (1) (i), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.2 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages each containing approximately 300 milligrams.

(b) The batch: A minimum of 5 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b) (1) (i) or (ii); except prepare the sample as follows: Place a representative portion of the sample (usually approximately 1.0 gram, accurately weighed) in a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake ointment and ether until homogeneous. Shake with a 25-milliliter portion of 0.1*N* hydrochloric acid. Remove the acid layer, and repeat the extraction with three 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the extractives, and make the proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. The sample may also be prepared by placing a representative portion of the sample (usually 1.0 gram, accurately weighed) in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8 (b) of this chapter.

**§ 148n.20 Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment is oxytetracycline hydrochloride and polymyxin B sulfate, in a suitable and harmless ointment base. Each gram of topical ointment contains the equivalent of not less than 30 milligrams of oxytetracycline base and not less than 10,000 units of polymyxin B sulfate. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2 (a) (1) (i), (vi), (vii), (viii), and (ix). The polymyxin B sulfate conforms to the requirements of § 148p.1 (a) (1) (i), (vi), (vii), and (viii) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter, except that in lieu of the statement "Caution: Federal law prohibits dispensing without prescription," the labeling of each package

shall bear information that the drug is for use only in the prevention of infections in minor cuts and abrasions, and a statement that use of the drug shall be discontinued and a physician consulted if signs of infection or irritation appear. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, moisture, and identity.

(*c*) The batch for oxytetracycline content, polymyxin content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $5.00 for each immediate container in the sample submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 148n.19(b) (1), except that its oxytetracycline content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in § 148p.3(b) (1) of this chapter, except that its polymyxin B sulfate content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

### § 148n.21 Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate eye and ear ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. It may contain cortisone, hydrocorisone, or a suitable ester of cortisone or hydrocortisone, and one or more suitable and harmless preservatives and stabilizing agents. Each gram of ointment contains the equivalent of not less than 5 milligrams of oxytetracycline base and not less than 10,000 units of polymyxin B sulfate. Its moisture content is not more than 1 percent. The oxytetracycline hydorchloride used conforms to the requirements of § 148n.2(a) (1) (i), (vi), (vii), (viii), and (ix). The polymyxin B sulfate used conforms to the requirements of § 148p.1(a) (1) (i), (vi), (vii), and (viii) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, moisture, and identity.

(*c*) The batch for oxytetracycline content, polymyxin content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) Polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $5.00 for each immediate container in the sample submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraphs (3) (ii) (*a*), (*b*), and (*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 148n.19(b) (1), except that its oxytetracycline content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin B content.* Proceed as directed in §148p.3(b) (1) of this chapter, except that its polymyxin B sulfate content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

### § 148n.22 Oxytetracycline hydrochloride ophthalmic oil suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride ophthalmic oil suspension is oxytetracycline hydrochloride in a suitable and harmless oil base. It may contain one or more suitable and harmless preservatives and stabilizing agents. Its potency is equivalent to not less than 10 milligrams of oxytetracycline base per milliliter. Its moisture content is not more than 1.0 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a) (1) (i), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b) (1) (i) or (ii), except prepare the sample as follows: Place a representative portion of the sample (usually 1.0 milliliter, accurately measured) in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

### § 148n.23 Oxytetracycline hydrochloride eye and ear suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride eye and ear suspension is oxytetracycline hydrochloride in a suitable and harmless oil base. It may contain cortisone, hydrocortisone, or a suitable ester of cortisone or hydrocortisone, and one or more suitable and harmless preservatives and stabilizing agents. Its potency is equivalent to not less than 5 milligrams oxytetracycline base per milliliter. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the requirements of § 148n.2(a) (1) (i), (vi), (vii), (viii), and (ix). Each other ingredient used, if its name is recognized

in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Oxytetracycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b) (1) (i) or (ii), except prepare the sample as follows: Place a representative portion of the sample (usually 1.0 milliliter, accurately measured) in a glass blending jar containing 1.0 milliliter of polysorbate 80 and 199 milliliters of 0.1*N* hydrochloric acid. Using a high-speed blender, blend the mixture for approximately 3 minutes and make proper estimated dilutions to the prescribed reference concentration in pH 4.5 buffer. The potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that the suspension is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

**§ 148n.24 Calcium di-oxytetracycline syrup.**

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium di-oxytetracycline syrup is syrup that contains calcium di-oxytetracycline, with or without one or more suitable and harmless buffer substances, suspending and stabilizing agents, flavorings, and preservatives, suspended in a suitable and harmless vehicle. It may contain glucosamine. Each milliliter contains a quantity of calcium di-oxytetracycline equivalent to not less than 25 milligrams of oxytetracycline base. Its pH is not less than 5.0 and not more than 8.0. The calcium di-oxytetracycline used conforms to the requirements of § 148n.3(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Calcium di-oxytetracycline used in making the batch for potency, toxicity, moisture, and calcium content.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The calcium di-oxytetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148n.1 (b) (1) (i) or (ii), except prepare the sample as follows: Transfer an appropriate aliquot (usually from 1.0 milliliter to 5.0 milliliters) to a 100-milliliter volumetric flask and make to mark with 0.1*N* hydrochloric acid. Withdraw an aliquot from the volumetric flask and dilute to the prescribed reference concentration using pH 4.5 buffer. Its content of oxytetracycline is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *pH.* Using the undiluted sample, proceed as directed in § 141a.5(b) of this chapter.

All interested persons are invited to present their views in writing regarding this proposal to the Hearing Clerk, Department of Health, Education, and Welfare, Washington 25, D.C., within 30 days from the date of publication of this notice in the FEDERAL REGISTER. Such views and comments should be filed preferably in quintuplicate.

Proposals for the certification of various other antibiotic drugs will be published in the immediate future.

Dated: April 9, 1963.

GEO. P. LARRICK,
*Commissioner of Food and Drugs.*

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